

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
3 June 2004 (03.06.2004)

PCT

(10) International Publication Number  
**WO 2004/046730 A3**

(51) International Patent Classification<sup>7</sup>: **G01N 33/94**,  
A61K 47/48, C07G 11/00, C07K 14/00, G01N 33/58,  
33/533

(21) International Application Number:  
PCT/IB2003/005430

(22) International Filing Date: 27 October 2003 (27.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0224872.2 25 October 2002 (25.10.2002) GB  
0229640.8 20 December 2002 (20.12.2002) GB  
0307379.8 31 March 2003 (31.03.2003) GB

(71) Applicant (for all designated States except US): **SENSE  
THERAPEUTIC LIMITED** [GB/GB]; Unit 4, The  
Switchback, Gardner Road, Maidenhead, Berkshire SL6  
7RJ (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HART, Darren**  
[GB/GB]; c/o Sense Therapeutic Limited, Unit 4, The

Switchback, Gardner Road, Maidenhead, Berkshire  
SL6 7RJ (GB). **GODBER, Ben** [GB/GB]; c/o Sense  
Therapeutic Limited, Unit 4, The Switchback, Gardner  
Road, Maidenhead, Berkshire SL6 7RJ (GB). **BLACK-  
BURN, Jonathan, M.** [GB/GB]; c/o Sense Therapeutic  
Limited, Unit 4, The Switchback, Gardner Road, Maid-  
enhead, Berkshire SL6 7RJ (GB). **MCANDREWS, Mike**  
[GB/GB]; c/o Sense Therapeutic Limited, Unit 4, The  
Switchback, Gardner Road, Maidenhead, Berkshire SL6  
7RJ (GB).

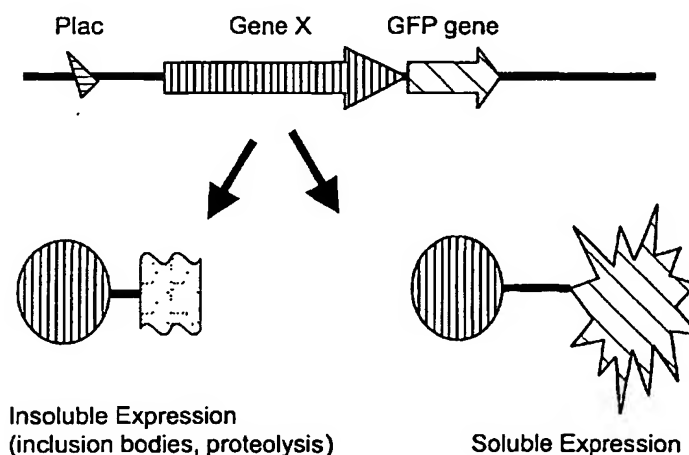
(74) Agent: **MACLEAN, Martin, R.**; Mathys & Squire, 100  
Gray's Inn Road, London WC1X 8AL (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE,  
GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,  
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,  
MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT,  
RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR,  
TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: **USES OF BLE PROTEINS AND ANTIBIOTICS FROM THE BLEOMYCIN FAMILY**



(57) Abstract: The present invention provides use of a protein conjugate comprising a "ble" protein, which has specific binding properties. The protein conjugates are capable of binding reversibly to an antibiotic from the bleomycin family, which property is exploited in a variety of immobilisation methods. In preferred aspects of the invention, the conjugates are used as markers for protein expression and/or folding, or for affinity tagging. The present invention also provides a probe comprising an array of an immobilised antibody from the bleomycin family, which acts as an analyte capture moiety. In another aspect, a purification media is provided, which comprises an antibiotic of the bleomycin family as an analyte capture moiety. Also provided is a method for generating soluble forms of an insoluble protein by expressing the protein as a "ble" fusion protein and selecting in the presence of an antibiotic from the bleomycin family. In a further aspect, the "ble" protein is expressed as a fusion protein in a cell into which is introduced a labelled antibiotic of the bleomycin family, thereby allowing identification of the cellular localisation of the protein.



Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,  
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Published:**

— *with international search report*

**(88) Date of publication of the international search report:**

2 September 2004

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 03/05430

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/94 A61K47/48 C07G11/00 C07K14/00 G01N33/58  
G01N33/533

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, PAJ, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BENNETT R P ET AL: "Fusion of green fluorescent protein with the Zeocin-resistance marker allows visual screening and drug selection of transfected eukaryotic cells" BIOTECHNIQUES, EATON PUBLISHING, NATICK, US, vol. 24, no. 3, March 1998 (1998-03), pages 478-482, XP002114401 ISSN: 0736-6205 figure 1 the whole document ----- -/--	1,2,5

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

\*A\* document member of the same patent family

Date of the actual completion of the international search

24 May 2004

Date of mailing of the international search report

03/06/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Jenkins, 6

# INTERNATIONAL SEARCH REPORT

national Application No  
PCT/IB 03/05430

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/27327 A (SENSE PROTEOMIC LTD ; SAMADDAR MITALI (GB); BLACKBURN JONATHAN MICHAEL) 4 April 2002 (2002-04-04) the whole document pages 15-17 claim 9	3,4
X	US 5 420 228 A (MISTRY JEHangIR S ET AL) 30 May 1995 (1995-05-30) the whole document claims 5,6	29-32
X	CALMELS THIERRY P G ET AL: "Nuclear localization of bacterial Streptoalloteichus hindustanus bleomycin resistance protein in mammalian cells" MOLECULAR PHARMACOLOGY, vol. 44, no. 6, 1993, pages 1135-1141, XP008030691 ISSN: 0026-895X the whole document page 1139, column 1, paragraph 2	29-32
A	US 6 027 900 A (GROSSMAN ARTHUR R ET AL) 22 February 2000 (2000-02-22) the whole document	33-35

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 03/05430

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0227327	A	04-04-2002	AU 7994801 A BR 0113330 A CA 2420415 A1 EP 1326970 A2 WO 0227327 A2 GB 2384779 A US 2003073811 A1	08-04-2002 30-12-2003 04-04-2002 16-07-2003 04-04-2002 06-08-2003 17-04-2003
US 5420228	A	30-05-1995	NONE	
US 6027900	A	22-02-2000	WO 9739106 A1	23-10-1997